SENESCENCE, FLUORESCENCE AND CRUSTACEAN AGE DETERMINATION

Assessment of the age of individuals is currently a significant problem in studies of crustacean population dynamics, specifically the management of commercially important crustacean stocks, which are coming under increasing threat of over exploitation.

Several methods have been tried for assessing age in crustaceans, but all are severely restricted. The most widely used method, that involving prediction of age from body size, based on growth rates determined from modal analysis, tag recapture programs or laboratory rearing, suffers a fundamental problem — variability of growth rate between individuals. Individuals of the same chronological age, growing in the same environmental regime, may sometimes differ in their size by an order of magnitude. This phenomenon clearly limits the usefulness of body size as an indicator of age. The wide application of this method of age prediction reflects the need for, but lack of, a suitable alternative. Thus, new approaches to crustacean age determination are essential. Ettershank (1983) attempted to use the intensity of extracted chloroform-soluble fluorescence to age field-captured Antarctic krill, *Euphausia superba*. Similar extractable fluorescence had been shown to accumulate linearly with age in insects and was thought to be derived from the universally occurring lipofuscin age-pigment.

Subsequently however, work by the present author and others (Nicol, 1987; Sheehy and Ettershank, 1989; Sheehy and Roberts, in press), some of which was on crustaceans of known age, revealed some serious flaws in the methods used by Ettershank and previous workers. Most importantly, 'lipofuscin-like' extractable fluorescence did not appear to be derived from lipofuscin and its intensity bore little relationship to age. Recently, prominent workers in the broader field of gerontology have been voicing serious doubts about the biochemical extraction method for lipofuscin determination (Sohal, 1987).

Clearly, the potential of lipofuscin as an index of age in crustaceans required reassessment.

Methods and Results

Fluorescent morphological lipofuscin was identified in histological sections from a wide range of crustaceans, including several of economic importance (Sheehy, 1989, 1990a). Alternative quantification techniques were developed. Using fluorescence microscopy and new image analysis methods (Sheehy, 1989, 1990b), conclusive evidence of the physiological age dependence of lipofuscin accumulation in the Crustacea was obtained for the first time. In laboratory reared freshwater crayfish, *Cherax quadricarinatus*, of precisely known chronological age, lipofuscin quantities in the base of the olfactory lobe cell mass of the brain were superior predictors of chronological age ($r = 0.96$) to morphometric parameters, such as carapace length, normally used for this purpose. While carapace length was able to place only 51% of the experimental individuals into their correct age class, lipofuscin volume fraction correctly placed 93% of the same individuals. Lipofuscin volume fractions were similar in the olfactory lobes of crayfish of similar chronological age despite a wide disparity in body size and weight.

Discussion

The present results suggest that lipofuscin has significant potential as an index of crustacean age. Although lipofuscin accumulates with physiological age, rather than strict chronological age, and its accumulation is affected by environmental parameters such as temperature (Sheehy, 1990b), growth is also influenced by environmental factors. At this stage, there is no reason to believe that lipofuscin would not be a superior predictor of age to body size under variable field conditions. Early results suggest that it may be possible to predict absolute chronological age of field animals using relatively simple laboratory established models incorporating ambient temperature (Sheehy, 1990b).

Literature Cited

Ettershank, G. 1983. Age structure and cyclical annual size change in the Antarctic krill, *Euphausia superba* Dana. Polar Biology 2: 189–193.

Nicol, S. 1987. Some limitations on the use of the lipofuscin ageing technique. Marine Biology 93: 609–614.

Sheehy, M.R.J. 1989. Crustacean brain lipofuscin: an examination of the morphological pigment in the freshwater crayfish *Cherax cuspidatus* (Crustacea: Parastacidae). Journal of Crustacean Biology 9(3): 387–391.

1990a. The widespread occurrence of fluorescent morphological lipofuscin in the crustacean brain. Journal of Crustacean Biology 10(4): 613-622.

1990b. Individual variation in, and the effect of rearing temperature and body size on, the concentration of fluorescent morphological lipofuscin in the brains of freshwater crayfish, Cherax cuspidatus (Crustacea: Parastacidae). Comparative Biochemistry and Physiology 96(A): 281-286.

Sheehy, M.R.J. and Ettershank, G. 1989. Solvent extractable, age pigmentlike autofluorescence and its relationship to growth and age in the waterflea *Daphnia carinata* King. Australian Journal of Zoology 36: 611-625.

Sheehy, M.R.J. and Roberts, B. In Press. An alternative explanation for discrepancies in lipofuscin fluorescence data from insects, crustaceans and other aquatic species. Experimental Gerontology.

Sohal, R.S. 1987. Quantification of lipofuscin: a critique of the current methodology. Advances in the Biosciences 64: 85–91.

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